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Variability of secondary metabolites from leaves of *Ziziphus mauritiana* obtained from different locations in Sumbawa, Indonesia

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Abstract. *Nairfana I, Nikmatullah A, Sarjan M, Tandeang A. 2022. Variability of secondary metabolites from leaves of* Ziziphus mauritiana *obtained from different locations in Sumbawa, Indonesia. Biodiversitas 23: 4948-4957.* Bidara (*Ziziphus mauritiana* L.) is an evergreen shrub that grows in many parts of the world, and is an important plant in arid and semi-arid regions for the environment, food, and health. The plant has been used traditionally as medicine, and more recently, growing scientific evidence demonstrated the active compounds of *Z. mauritiana* leaves. However, little is known about the plant's secondary metabolite profiles in different environments. This research aimed to characterize the secondary metabolites profile of *Z. mauritiana* leaf obtained from the different habitats of semi-arid Sumbawa Island, Indonesia, and to evaluate their relationship based on cluster analysis of the secondary metabolite diversity. The leaf samples were collected from *Z. mauritiana* plants obtained in 3 districts on Sumbawa Island: West Sumbawa (SB), Sumbawa (KS), and Dompu (DP) Districts, each from different ecosystems: lowland coastal/savanna, lowland near residential areas, and mid-highland areas of residential or forest-edge. The leaf samples were extracted by maceration with multilevel extraction using solvents with decreased polarity (h-hexane, ethyl acetate, ethanol 70%, methanol, and distilled water, respectively), followed by phytochemical screening for the presence of alkaloid, flavonoid, saponin, and phenolic. The results showed variation in the phytochemical components of *Z. mauritiana* leaves obtained from a different environment, with secondary metabolites of DP leaves being more diverse than KS and other SB. All samples contained polar alkaloids and flavonoids, while the DP1 samples also contained non-polar flavonoids. The SB1 and DP samples also contained saponin. Interestingly, phenolic was only detected in the polar extracts of DP1 leaves. Cluster analysis based on the secondary metabolites profile distinguished the SB1 and DP1 *Z. mauritiana* from others. Further study is required to understand if the variation is resulted from *Z. mauritiana* adaptation to a different environment or also due to genetic variation of the *Z. mauritiana* in Sumbawa Island.

Keywords: Bidara**,** ecosystem**,** phytochemical, semi-arid, *Ziziphus mauritiana*

INTRODUCTION

Bidara (*Ziziphus mauritiana* L.) plant is found in various parts of the world, including Africa (Stadlmayr et al. 2013), Australia and Fiji (Dhileepan 2017), India (Prakash et al. 2010), Pakistan (Sharif et al. 2019), China (Uddin et al. 2021) and Malaysia (Jaelani et al. 2020). In Indonesia, *Z. mauritiana* grows wild in areas up to 400 m above sea level, including in areas with extreme temperatures and dryness (Gunawan et al. 2017; Zandalinas et al. 2017). The local names for *Z. mauritiana* are widara (Java, Sunda), rangga (Bima), kalanga (Sumba), bekul (Bali), kom (Kupang) and goal (Sumbawa). *Z. mauritiana* has long been used as traditional medicine. In some countries, *Z. mauritiana* treats wounds, asthma, diarrhea, fever, abscesses, and swelling (Mishra et al. 2011; Nyanga et al. 2013; Delphine et al. 2017; Moeini et al. 2020; Shady et al. 2022). Scientific studies have shown that the *Z. mauritiana* plant contains compounds that act as an antioxidant (Dahiru and Obidoa 2007; Bhatia and Mishra 2009; Gupta 2018), antimicrobial and antileishmanial (Abalaka et al. 2010; Hammi et al. 2022), and antidiabetic (Jarald et al. 2009). The compound from the *Z. mauritiana* plant is also reported to have the ability to suppress the development of cancer cells (Zazouli et al. 2022).

The use of *Z. mauritiana* continues to grow as scientific evidence demonstrates diverse bioactive ingredients of the plants (Dahiru and Obidoa 2007; Bhatia and Mishra 2009; Jarald et al. 2009; Delphine et al. 2017; Askur and Ganning 2021; Hammi et al. 2022; Zazouli et al. 2022). Most of the analyses reported the utilization of wild or cultivated *Z. mauritiana* plants in a specific study area, but little is known about the secondary metabolites' profiles of the *Z. mauritiana* plant grown in a different environment. The information will be beneficial to standardize the quality of raw materials for cultivation and breeding purposes, as *Z. mauritiana* is reported to adapt to the diverse environment, including the area with high temperatures, arid and semi-arid regions, alkaline conditions, very shallow soils, gravel plains, sand dunes, and rocky areas (Saran et al. 2007). Initial observation indicates that in Sumbawa Island, *Z. mauritiana* exists in various ecosystems, including the coast, yard, garden, rice field, edge of a forest, and savanna, in both the lowlands and medium plains. The distribution of *Z. mauritiana* on this island is quite wide and is found on various types of land with various

environmental conditions. This difference in growth habitat can affect *Z. mauritiana* plant growth and development as well as the type and levels of secondary metabolites. Plants will produce more secondary metabolites when subjected to abiotic stresses such as water stress (Awasthi and More 2009; Chiou et al. 2020). Besides being influenced by the growing environment, the type and levels of secondary metabolites extracted from a plant are influenced by the type of solvent used. Selecting the right solvent can increase extraction efficiency (Nairfana et al. 2020). Many factors need to be considered in selecting suitable solvents, including the price, ease of obtaining them, physical and chemical stability, neutrality, ease of evaporation, and reactivity with the extracted substances (Naderi and Babadagli 2015). Therefore, it is necessary to optimize the extraction of secondary metabolites from *Z. mauritiana* leaf by varying the solvents with various polarities in the extraction process to extract different groups of compounds. The data will provide secondary metabolite profiles of the *Z. mauritiana* plants from different environments and different polarities of the solvents. The information might be further used to evaluate the variability of the *Z. mauritiana* plants grown on Sumbawa Island.

This study examined the diversity of secondary metabolites in the *Z. mauritiana* plant's leaves grown in different Sumbawa Island habitats and used solvents with different polarities. The secondary metabolite variations were then used to estimate the relationship between *Z. mauritiana* plants growing on Sumbawa Island. This finding is very important for standardizing raw materials used for medicine, cosmetics, and functional food production from *Z. mauritiana*, as well as for conservation, cultivation, and plant breeding.

MATERIALS AND METHODS

Study area

This research was conducted in 3 out of 5 districts on Sumbawa Island, Indonesia, designated as West Sumbawa (SB), Sumbawa (KS), and Dompu (DP) Districts. These three districts were chosen to represent Sumbawa Island areas with different climates based on their annual rainfall: 750-1000 mm/year (Dompu), 1000-1250 mm/year (KS), and 1250-1500 mm/year (SB) (Kementerian Pekerjaan Umum dan Perumahan Rakyat 2016). In each district, 3 sampling locations were determined purposively: one sub-district in the lowlands (area close to the beach or in the lowland savanna), one sub-district in the residential area or forest edge of the lowland, and one sub-district in the medium-high plains - in the yard or forest-edge. In each sub-district, 1-3 villages from the same district were designated as sampling locations based on the presence of *Z. mauritiana* plants (population more than 5), and at each location, 3 plants were assigned and sampled. Plants selected as the samples were plants more than 3 years old (based on information from residents) and were in the generative phase. Figure 1 shows a demographic map of Sumbawa Island with the sampling districts in this study.

Habitat and environmental parameters of sampling sites

The habitat and environmental values for all sample locations are listed in Table 1, and the representative sample locations are visualized in Figure 2.

Procedures

Sample collection

Leaf samples were taken from *Z. mauritiana* plants at each location from May to July 2022. First, fully developed-green leaves (excluding the top of the leaf and at the base of the branch) were harvested, placed in zip-lock plastic bags, labeled, and taken to the Food and Agroindustry Laboratory, Faculty of Agricultural Technology, Universitas Teknologi Sumbawa, Indonesia (usually a day on the road). Upon arrival in the laboratory, the samples were immediately stored in the refrigerator (at *ca.* 4-7°C) until used for phytochemical screening (storage time 1- 3 weeks).

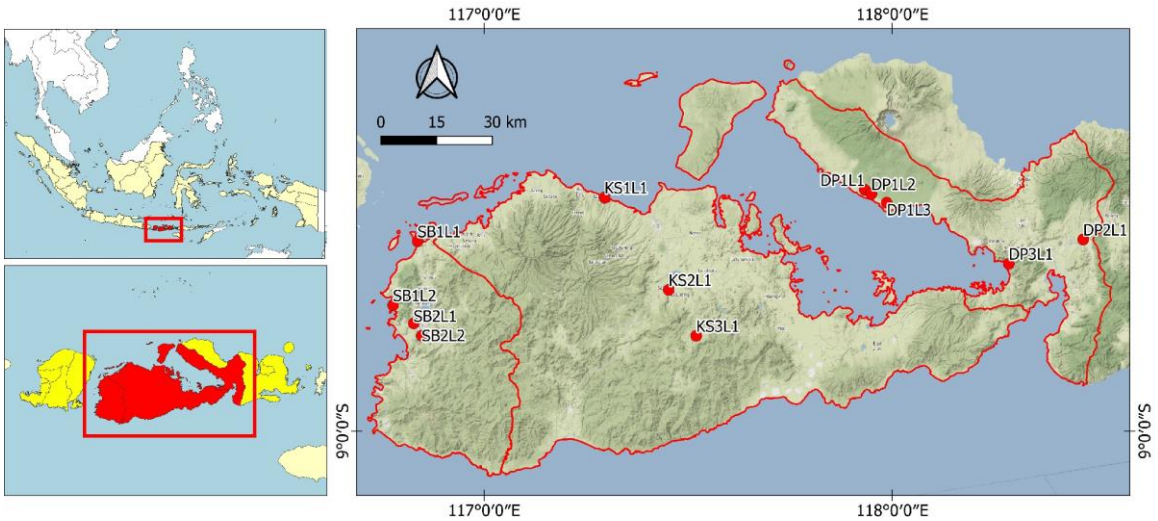

Figure 1. Sampling districts of *Ziziphus mauritiana* in Sumbawa Island, Indonesia, reported in this study (SB: Sumbawa Barat; KS: Sumbawa; DP: Dompu)

Table 1. Environmental parameters of *Ziziphus mauritiana* samples in each sampling site

Sample	Location	Habitat	Date collected	Altitude (m a.s.l.)	Latitude/ Longitude
KS	**Sumbawa**				
KS1L1	Batu Gong	Coastal	24/6/22	5	-8.431773; 117.295103
KS2L1	Moyo Hulu	Lowland	22/6/22	120	-8.656250; 117.452178
KS3L1	Lantung	Medium-Highland	22/6/22	720	-8.768141; 117.519681
SB	**Western Sumbawa**				
SB1L1	Poto Tano	Coastal	18/6/22	5	-8.53803; 116.837219
SB1L2	Kertasari	Coastal	24/6/22	5	-8.693576; 116.776946
SB2L1	Taliwang -1	Lowland	23/6/22	72	-8.737756; 116.827515
SB2L2	Taliwang-2	Lowland	23/6/22	84	-8.737756; 116.827515
DP	**Dompu**				
DP1L1	Doropeti, Pekat	Coastal Savanna	19/6/22	15	-8.412919; 117.932794
DP1L2	Pekat, Tambora-1	Coastal savanna	19/6/22	17	-8.423236; 117.948598
DP1L3	Pekat, Tambora-2	Coastal savanna	19/6/22	18	-8.444452; 117.986340
DP2L1	Dompu Village	Lowland	21/6/22	120	-8.533695; 118.467708
DP3L1	Nanga Tumpu	Medium-Highland	21/6/22	360	-8.592691; 118.2855513

Figure 2. Representation of *Ziziphus mauritiana* trees in some sampling sites showing different ecosystem in each sampling sites: forest edge (SB2L1), savanna (DP1L2), or residential area (KS3L1)

The samples come from a wide range of habitats (from coastal lands with open plains, coastal savannas, and lowlands, including trees that grew in the backyard or near dry-land paddy fields and mid-land with dense forest). The weathers on the day of the collection are mainly sunny. Four different types of secondary metabolites (alkaloid, flavonoid, saponin, and phenolic compounds) were observed. Extraction was carried out by maceration and multilevel extraction based on the degree of polarity of the solvents.

Multilevel extraction

Extraction was carried out using the maceration method (Cujic et al. 2016). Fresh *Z. mauritiana* leaf samples were ground using a pestle and mortar and then extracted using solvents with increasing polarity. The solvents were n-hexane, ethyl acetate, 70% ethanol, absolute methanol, and distilled water. The sample:solvent ratio was 1:10 (Nairfana et al. 2020). The first maceration was done by soaking 10 g of *Z. mauritiana* leaf powder with 100 ml of n-hexane (1:10; w/v) for 24 hours while occasionally stirring (every 6 hours). After 24 hours, the residue was separated from the filtrate using filter paper, and the pulp was dried in an oven at 50°C. Phytochemical screenings were performed on the filtrate obtained. The dry residue was then macerated again following the same procedure using ethyl acetate, followed by 70% ethanol, absolute methanol, and distilled water.

Phytochemical screening

Alkaloid test

Typically, 1 mL of liquid extract was mixed with 5 mL of chloroform and 2 drops of NH4OH using a dropping pipette and then transferred into a test tube. Next, an aliquot of 6 mL H_2SO_4 was added, and the reaction was gently mixed, and then, 2 drops of Dragendorf reagent using a dropping pipette were added. The reaction was left to stand for 1 minute and then examined. The presence of alkaloids is indicated by brown or orange colored deposits (Raal et al. 2020).

Flavonoid test

A total of 1 mL of liquid extract was transferred into a test tube, and then 1 mL of 10% Pb acetate was added and

the tube was shaken gently. The reaction was left to stand for 1 minute and then examined. A change indicates a positive reaction in the color of extract to yellowish brown (Patie et al. 2020).

Saponin test

Hot distilled water was added to the liquid extract, and the mixture was shaken vigorously for 1 minute. If foam appeared, 5 drops of HCl 2M solution using a dropping pipette were added to the foam, and the reaction stood for 10 minutes. The foam produced should be stable for 10 minutes with a height of 1-3 cm, then the extract is categorized as positive for the saponin test (Patie et al. 2020).

Phenolic test

A total of 1 mL of liquid extract was transferred into a test tube and then mixed gently with 1 mL of 1% $FeCl_3$ solution. Positive test results for the presence of phenolic compounds are indicated by the formation of green, red, purple, or solid black colors (Patie et al. 2020).

Data analysis

The data obtained are presented in tables and dendrogram diagrams of the phytochemical diversity of *Z. mauritiana* leaf extract. Data processing was performed with Microsoft Excel (Ver. 2003) and NTSYS PC (Ver 2. 10e).

RESULTS AND DISCUSSION

All the secondary metabolites observed from each sampling location are presented in Tables 2-4, while the color changes during the phytochemical screening of each secondary metabolite are presented in Figures 3-7.

Distilled water and methanol could dissolve alkaloid, flavonoid, saponin, and phenolic from most of the extracts, with more positive results, were obtained in the methanol extracts than in distilled water. On the other hand, fewer positive results were obtained in semi-polar and non-polar extracts. The semi-polar ethyl acetate could dissolve alkaloid and flavonoid in DP1L3-3 and DP1L1 samples while only leaf from DP1L1-, DP1L2-1, DP1L2-2, DP1L3-1, DP1L3-2, and DP1L3-3 contained flavonoid that dissolved by non-polar n-Hexane. Overall, the results showed that *Z. mauritiana* leaves obtained from DP-1 had more diverse secondary metabolites than *Z. mauritiana* leaves from other regions. Secondary metabolites are produced more by plants when plants are under stress conditions (Zandalinas et al. 2007). Secondary metabolites are chemical compounds that generally have bioactivity, capabilities, and function as plant protectors from pests and diseases for the plant itself or its environment. It is known that plants exposed to high intensity of the sunlight can produce more secondary metabolites than plants grown in shades. It is noted that *Z. mauritiana* plants grow well in locations exposed to lots of sunshine, and *Z. mauritiana* was the dominant tree in the savanna in lowland Dompu (DP1) and West Sumbawa (SB1).

The phytochemical screening result in Table 2 showed that alkaloid compounds were presented in most samples when extracted with polar to semi-polar solvents. These were seen from the formation of dark green solutions with orange sediments at the bottom of the test tubes. In the alkaloid test with Dragendorff's reagent, nitrogen forms a coordinate covalent bond with K^+, a metal ion. The reaction in Dragendorff's test is shown in Figure 3.

In contrast with the polar solvents, less polar solvents such as n-hexane and a few samples of ethyl acetate (apart from some samples from DP) resulted in a negative alkaloid test. This was characterized by the absence of sediments at the test tubes' bottom. The documentation of color changes for the negative alkaloid test is shown in Figure 4.

Table 2. Phytochemical test results of *Ziziphus mauritiana* leaf extract from SB extracted with different solvents

Samples	Secondary metabolites	Solvents				
		n-Hexane	Ethyl acetate	Ethanol	Methanol	Distilled water
SB1L1-2	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	+
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	-	-
SB1L1-3	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	+
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	+	-
SB1L2-1	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	+
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	-	-
SB1L2-2	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	+
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	-	-
SB1L2-3	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	+
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	-	-
SB2L1-1	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-
SB2L1-2	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-
SB2L2-1	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	-	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-
SB2L2-2	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	-	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-
SB2L2-3	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	-	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-

Table 3. Phytochemical test results of *Ziziphus mauritiana* leaf extract from KS extracted with different solvents

Samples	Secondary metabolites	Solvents				
		n-Hexane	Ethyl acetate	Ethanol	Methanol	Distilled water
KS1L1-1	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	+
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	-	-
KS1L1-2	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	+
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	+	+
KS1L1-3	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	+
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	+	+
KS2L2-1	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	-	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-
KS2L2-2	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	-	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-
KS2L2-3	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-
KS3L1-1	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-
KS3L1-2	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-
KS3L1-3	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	-	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-

Alkaloids are basic compounds in the presence of nitrogen atoms in their structure. Alkaloids have a characteristic of solubility in organic solvents. This group of compounds is easily soluble in alcohol and slightly soluble in water. Alkaloid salts are usually soluble in water. Alkaloids are polar (Patie et al. 2020), so they can be extracted with polar and semi-polar solvents such as distilled water, ethanol, and methanol. According to Vafaei and Abdollahzadeh (2015), alkaloid salts differ from free alkaloids in alkaline form. Alkaloids in the basic form are usually insoluble in water but easily soluble in organic solvents (such as benzene, ether, chloroform), while in their salt form, alkaloids are easily soluble in polar solvents. This shows that the content of alkaloid compounds in *Z. mauritiana* leaf extract is an alkaloid compound in the form of its salt because the positive reaction is shown in polar solvents.

Table 4. Phytochemical test results of *Ziziphus mauritiana* leaf extract from DP extracted with different solvents

Samples	Secondary metabolites	Solvents				
		n-Hexane	Ethyl acetate	Ethanol	Methanol	Distilled water
DP1L1-1	Alkaloid	-	-	+	+	+
	Flavonoid	+	-	-	+	+
	Saponin	-	-	-	+	+
	Phenolic	-	-	-	+	+
DP1L1-2	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	+
	Saponin	-	-	-	+	+
	Phenolic	-	-	-	+	-
DP1L1-3	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	+	+	+
	Saponin	-	-	+	+	+
	Phenolic	-	-	-	+	+
DP1L2-1	Alkaloid	-	-	+	+	+
	Flavonoid	+	-	+	+	+
	Saponin	-	-	-	+	+
	Phenolic	-	-	-	+	-
DP1L2-2	Alkaloid	-	-	+	+	+
	Flavonoid	+	-	+	+	+
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	+	-
DP1L2-3	Alkaloid	-	-	+	+	+
	Flavonoid	-	-	-	+	+
	Saponin	-	-	-	+	+
	Phenolic	-	-	-	+	-
DP1L3-1	Alkaloid	--	-	+	+	+
	Flavonoid	+	-	-	+	+
	Saponin	-	-	-	+	+
	Phenolic	-	-	-	+	-
DP1L3-2	Alkaloid	-	-	+	+	+
	Flavonoid	+	-	-	+	+
	Saponin	-	-	-	+	+
	Phenolic	-	-	-	-	-
DP1L3-3	Alkaloid	-	-	+	+	+
	Flavonoid	+	+	+	+	+
	Saponin	-	-	-	+	+
	Phenolic	-	-	-	-	-
DP2L1-1	Alkaloid	-	-	+	+	+
	Flavonoid	-	+	+	+	-
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	-	-
DP2L1-2	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	+	+	-
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	-	-
DP2L1-3	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	-
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	-	-
DP3L1-1	Alkaloid	-	-	+	+	+
	Flavonoid	-	-	-	+	-
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	-	-
DP3L1-2	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	+	-
	Saponin	-	-	-	-	-
	Phenolic	-	-	-	-	-
DP3L1-3	Alkaloid	-	-	-	+	+
	Flavonoid	-	-	-	-	-
	Saponin	-	-	-	+	-
	Phenolic	-	-	-	-	-

Figure 3. Phytochemical screening results of positive alkaloid compounds with Dragendorff's reagent. A. Ethanol, B. Methanol, C. Distilled water

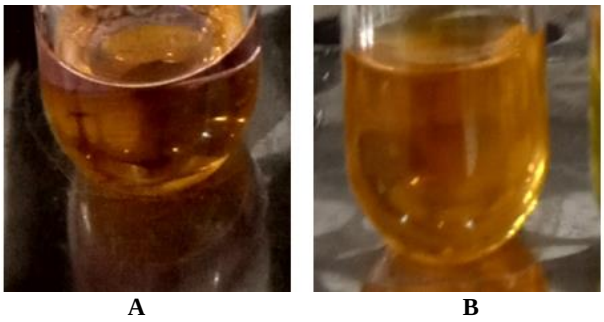

Figure 4. Phytochemical screening results of negative alkaloid compounds with Dragendorff's reagent. A. n-Hexane, B. Ethyl acetate

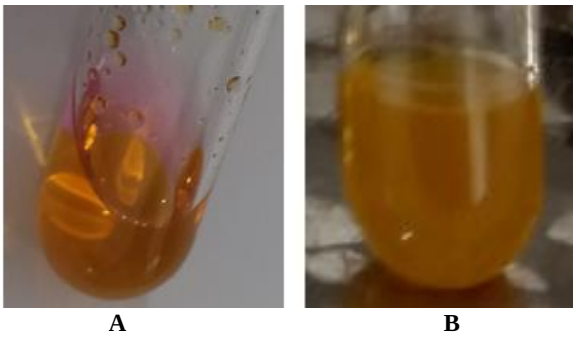

Figure 5. Phytochemical screening results of positive flavonoid when extracted with methanol. A. SB1L12, B. DP1L12

Similar to alkaloids, the presence of flavonoids was also found in almost all plant samples extracted with polar solvents, such as distilled water and methanol. Positive results of flavonoid testing were determined by the presence of red, yellow, or orange color in the sample after adding Mg and HCl powder. Flavonoid compounds are polar, so a polar solvent is needed (Gillespie and Paul 2001), and the flavonoids of *Z. mauritiana* leaves are soluble in distilled water, ethanol, and methanol. Among the three types of solvents that can extract these flavonoids, it is seen that methanol is the most effective solvent in this study, as almost all samples contain flavonoids after being extracted using methanol. Alcoholic compounds such as methanol and ethanol are excellent solvents for extraction because they can extract both polar and non-polar compounds. This is because methanol has two functional groups with different levels of polarity: a polar hydroxyl group and a non-polar alkyl group. Meanwhile, according to Arukwe et al. (2012), distilled water is a polar compound and can only extract polar compounds, so the flavonoid component has a lower solubility in water. Figure 5 shows the positive reaction of the flavonoid phytochemical test on several samples from the Dompu District, which were extracted using methanol.

The saponin test was carried out by hydrolysis of saponins in water. The appearance of foam indicates the presence of glycosides, which can form foam in water hydrolyzed to glucose. Positive results of saponin testing with foam formation were shown in the treatment with distilled water and methanol as solvents. The foam indicates the positive reaction formed no less than 10 minutes after shaking and is stable with adding 2M HCl (Patie et al. 2020). Meanwhile, in the treatment with ethyl acetate and n-hexane, foam formation did not occur, so the test could be considered negative because the solubility of the ethyl acetate and n-hexane samples was very low in the water, resulting in precipitation of the sample. According to Meshram et al. (2021), saponin compounds have polar (glycosyl groups) and non-polar (steroid or triterpenoid groups) so that they are surface active and when saponins are shaken with water, they undergo hydrolysis and form micelles. The micelle structure formed causes the polar groups to face out, and the non-polar groups face in so that it will look like foam. Figure 6 shows the positive test results for saponins in DP2L1 samples extracted using methanol.

Positive results of the phenol test with the occurrence of green to blackish green in the sample after the addition of 5% $FeCl_3$ were shown in all types of solvent treatments (Table 2). Phenol compounds have a hydroxyl group that can react with Fe^{3+} ions in a 5% $FeCl_3$ solution, forming black-green complex compounds (Cahen et al. 2021). From all leaf samples, only leaves from DP1 showed positive phenol compounds extracted using methanol from all leaf samples. Meanwhile, other samples that used methanol or other solvents showed negative reactions. Figure 7 shows a sample of DP1, which is positive for phenolic compounds.

In relationship, taxa are classified based on the similarities or dissimilarities between two or more taxa. So, plants in the same taxa may have similar morphology and biochemical content. The closer the relationship between two individuals, the greater the degree of similarity between the two individuals. In this study, the secondary metabolite profile of *Z. mauritiana* on Sumbawa Island was examined by phenetic analysis to characterize their relationship. The results of this test are presented as dendrograms which show the diversity of secondary metabolites as characters used as differentiators. The *Z. mauritiana* of Sumbawa Island is clustered into 2 main groups based on the diversity of the alkaloids in the *Z. mauritiana* leaves (Figure 8). Most SB and KS samples are in the same cluster (except for SB1L2-2 and SB1L2-3) and differ from DP2 and most of the DP1 samples.

Figure 6. Phytochemical screening results of positive saponin when extracted with methanol

Figure 7. One of the DP samples showed a positive phenolic reaction was seen to change to blackish green

All extracts' flavonoid and saponin profiles were also used to evaluate the relationship between each sample. Figure 9 shows the dendrogram of *Z. mauritiana* relationship based on the presence of flavonoid compounds. Similar to the alkaloid kindship, the *Z. mauritiana* plants were also separated into 2 major groups based on their flavonoid profile, and again most of the DP groups were separated with the KS/SB cluster. However, the saponin profile cluster was quite different from the flavonoid and alkaloid clusters. Although there were 2 major clusters of saponin in *Z. mauritiana* samples (Figure 10), the member of each cluster was mixed between the SB, KS, and DP.

The dendrogram of the phenolic compound was also developed (Figure 11). The dendrogram shows a similar pattern to the diversity of alkaloids and flavonoids with 2 groups of *Z. mauritiana* plants based on the diversity of alkaloid compounds in their leaves. The first cluster was divided into 2 groups, with KS/SB clustered together with DP1L1-3, DP1L2-1, DP1L2-2, DP1L3-1, and DP2L1.2, and differed from other DP.

Besides being influenced by the solvent used, the presence of secondary metabolites is also thought to be influenced by other factors, both internal and external factors. The genetic composition of the plant and the environmental factors such as sunshine and UV irradiation exposure (Ashraf et al. 2015; Delphine et al. 2017), altitude (Nyanga et al. 2013), latitude, climate, general state of weather throughout the year (Ashraf et al. 2015) and nutrient availability (Bhatia and Mishra 2009). It can be seen in Table 2 that there are several samples taken in the Dompu District that showed positive results on alkaloids, flavonoids, saponins, and phenolics when extracted with distilled water, methanol, and ethanol, while the presence of such compounds in all *Z. mauritiana* extracts from other samples were negative. Those differences might be due to different environmental conditions between Dompu District and Sumbawa/West Sumbawa District.

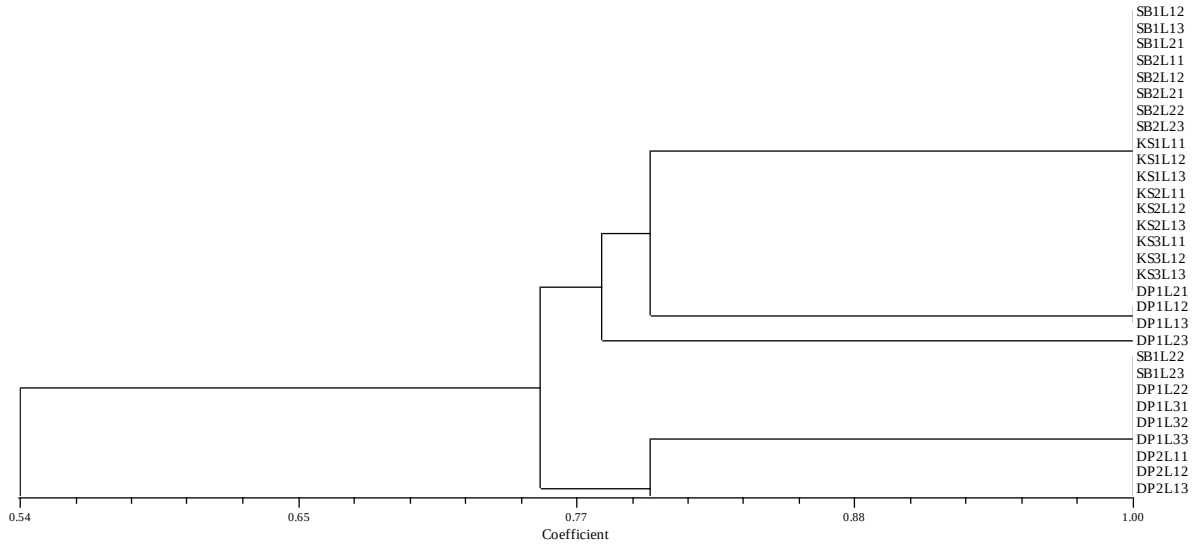

Figure 8. Dendrogram of alkaloid presence in *Ziziphus mauritiana* leaf extract of Sumbawa Island, Indonesia

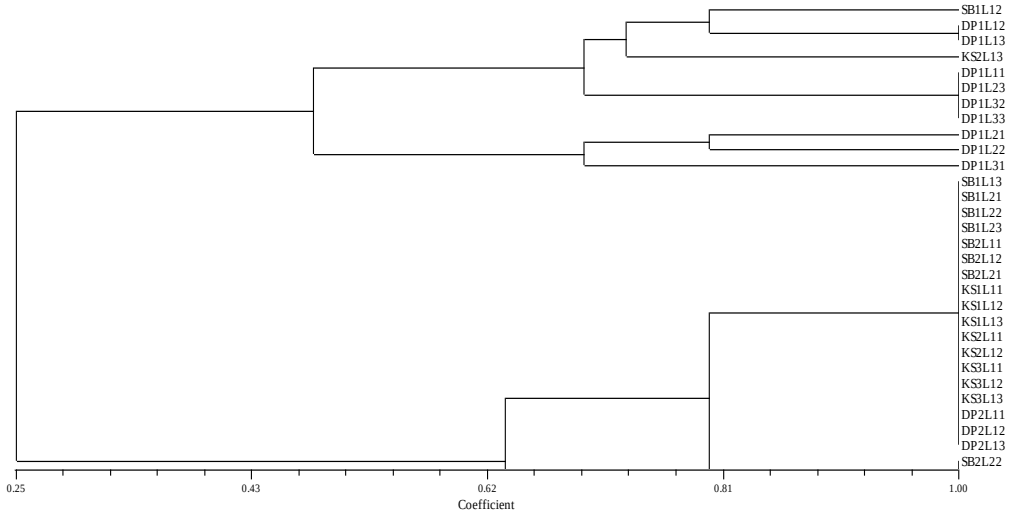

Figure 9. Dendrogram of flavonoid presence in *Ziziphus mauritiana* leaf extract from Sumbawa Island, Indonesia

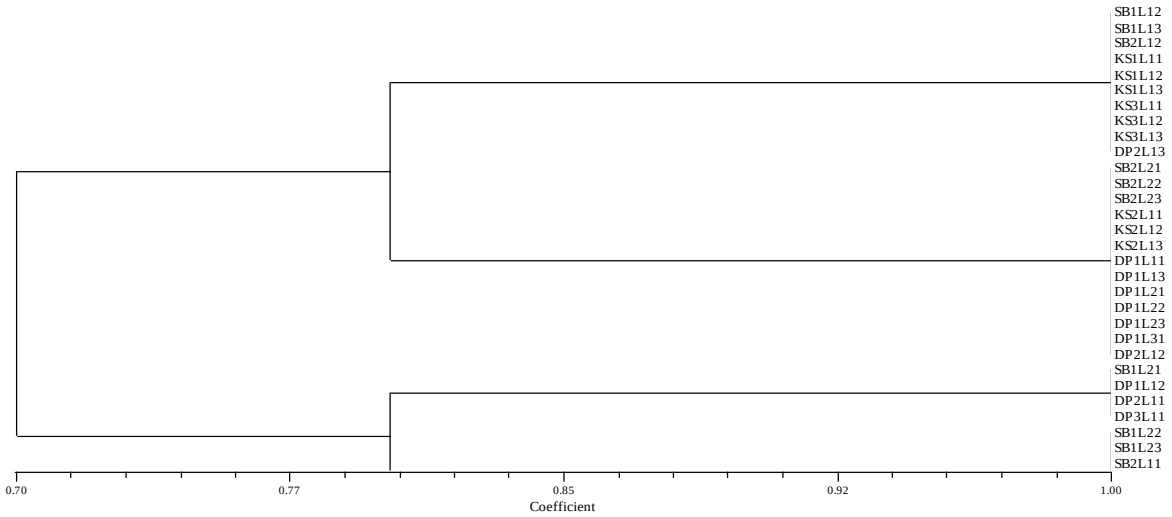

Figure 10. Dendrogram of saponin presence in *Ziziphus mauritiana* leaf extract from Sumbawa Island, Indonesia

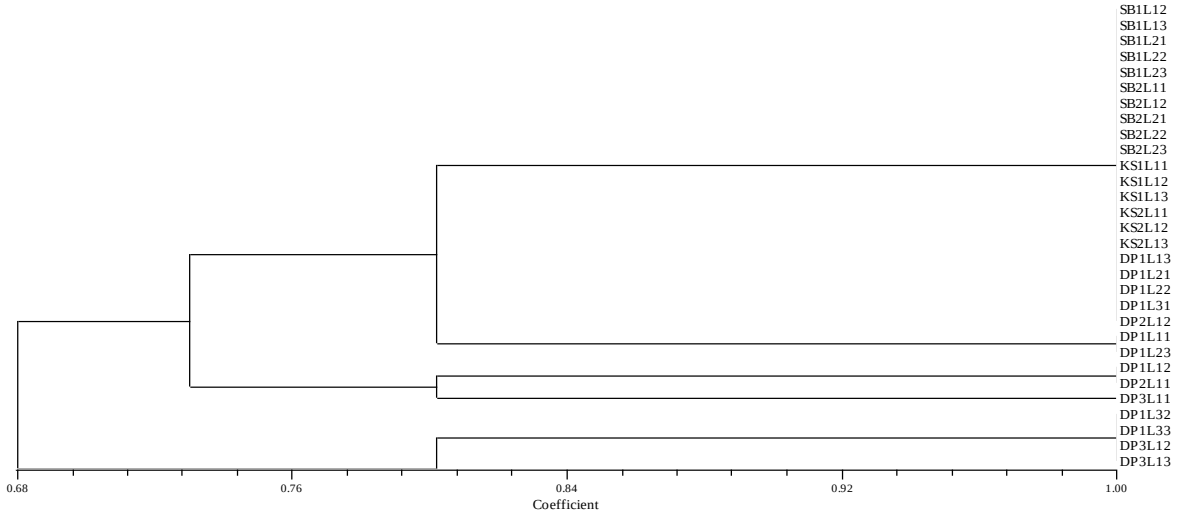

Figure 11 Dendrogram of phenolic presence in *Ziziphus mauritiana* leaf extract from Sumbawa Island, Indonesia

Dompu District is known to have less rainfall and is warmer than Sumbawa District and West Sumbawa District. The annual rainfall of Dompu (DP) was 750-1000 mm/year, while Sumbawa (KS) was 1000-1250 mm/year, and West Sumbawa (SB) was 1250-1500 mm/year (SB) (Kementerian Pekerjaan Umum dan Perumahan Rakyat 2016). Secondary metabolites are produced more by plants when plants are under stress conditions (Zandalinas et al. 2007). Secondary metabolites are chemical compounds that generally have bioactivity, capabilities, and function as plant protectors from pests and diseases for the plant itself or its environment. It is known that plants exposed to high intensity of sunlight can produce more secondary metabolites than plants grown in shades. It is noted that *Z. mauritiana* plants grow well in locations exposed to lots of sunshine, and *Z. mauritiana* was the dominant tree in the savanna in lowland Dompu (DP1) and West Sumbawa (SB1).

By testing the presence of secondary metabolites, it is known that, in general, alkaloids and flavonoids are compounds that are presented in all plants in this study. However, in this study, saponins and compounds were only found in plants obtained from SB and KS grown on the coast and savanna, as well as in all of the samples obtained in DP. Interestingly, phenolics were only detected in leaf extracts taken from savanna in Dompu District (DP1). The data indicate a distinct phytochemical secondary metabolite profile between the leaf of *Z. mauritiana* from drier places, particularly DP1, with other regions sampling regions. Cluster analysis was employed to distinguish the phytochemical secondary metabolite profile of the *Z. mauritiana* leaves taken from different environments in Sumbawa. In general, all phytochemical profiles were separated into 2 main clusters, with one cluster comprised of a group of plants that grow in Sumbawa and most West Sumbawa Districts and the second group comprised of plants that grow in Dompu District and coastal/savanna in West Sumbawa District. Therefore, cluster analysis based on the secondary metabolite profile may be used to determine the level of diversity in *Z. mauritiana* grown in a semi-arid region of Sumbawa Island. Further studies are required to explore if the diversity is due to external factors only or if there is also a genetic variation of *Z. mauritiana* in Sumbawa Island.

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